N$_2$ fixation in two Sesbania species and its transfer to rice (Oryza sativa L.) as revealed by $^{15}$N technology

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Summary. We used $^{15}$N technology to investigate N$_2$ fixation by Sesbania speciosa and Sesbania rostrata and its transfer to a lowland rice crop after incorporation of the Sesbania spp. into soil as green manure. During the first 50 days after establishment in November–December 1989, S. speciosa and S. rostrata produced 1126 and 923 kg dry matter ha$^{-1}$ respectively. They gathered 31 and 23 kg N ha$^{-1}$ respectively, of which 62%±5% and 55%±3% respectively, came from N$_2$ fixation. Both these species produced a greater biomass during September–October 1989, with S. rostrata producing more than S. speciosa. These results reflected differential responses by the plants to different day lengths at different times of the year. Furthermore, the dry matter yield and %N of $^{15}$N-labelled S. speciosa were smaller than those of the unlabelled plants, possibly due to inhibition of N$_2$ fixation in root nodules by the chemical N fertilizers added during labelling. These differences were not so pronounced in the stem-nodulated S. rostrata. The increased grain yield of rice fertilized with N in the form of chemical fertilizer or green manure was a result of an increased number of panicles per hill. The rice crop manured with S. speciosa produced a lower grain yield, with a lower grain weight than that manured with S. rostrata. This was due to a low uptake of soil N by rice manured with S. speciosa. Recovery of N from the green manure in rice straw with S. speciosa was significantly higher than from rice manured with S. rostrata, because of the higher applied N uptake by rice manured with the former.

Key words: Biological N$_2$ fixation – Sesbania speciosa – Sesbania rostrata – Green manure – N transfer – Wetland rice – $^{15}$N isotopic techniques

Many species of Sesbania are flood-tolerant and can be cultivated as green manure for lowland rice. However, the high photoperiodic sensitivity of the promising, stem-nodulating S. rostrata has restricted its use during short- day seasons (Becker et al. 1990). Results from field trials with S. rostrata, S. aculeata, S. sésban, and S. speciosa have indicated that S. speciosa is the most promising species for Sri Lanka in terms of leaf biomass production (Palm et al. 1988). In the report by Palm et al. (1988) S. speciosa in Sri Lanka was misidentified as S. sésban. Observations mad by Rudd (1992) together with closer examination of herbarium material made available by Dr. M. Jayasuriya, Curator, National Herbarium, Royal Botanic Gardens, Sri Lanka, have now confirmed that this species is S. speciosa and not S. sésban. S. speciosa is a naturalized plant that grows in the dry zones of Sri Lanka (Rudd 1992). Early evaluations of its use as a green manure for rice showed that this plant releases N rapidly during decomposition of its N-rich leaves (Palm et al. 1988). Therefore a basal application of inorganic-N fertilizer is not recommended when S. speciosa is incorporated into the soil just before rice is transplanted. So far there have been no quantitative estimates of the N$_2$ fixed by this green manure species.

In an intensively managed, two-crop cycle of rice, a legume for green manure can be grown only during a 40- to 60-day fallow period between the two rice-growing seasons. Furthermore, the green manure must be incorporated into the soil at least 10 days before the rice is transplanted. Under these circumstances, only efficient N$_2$-fixing green manures can make a major contribution to the total N requirement of the rice crop.

Most of the recent estimates of N$_2$ fixation by Sesbania spp. have been made either in pot experiments using the $^{15}$N isotope-dilution technique (Ndoye and Dreyfus 1988) or under laboratory conditions using the acetylene reduction assay (Becker et al. 1990). The $^{15}$N-dilution technique is based on the differential dilution of N absorbed from soil and from a labelled fertilizer by a N$_2$-fixing and a non-fixing plant, where atmospheric $^{14}$N being fixed in the former dilutes the $^{15}$N content. Early
workers used the difference method, which compares the total N content of a fixing and a non-fixing plant (Weber 1966; Ham et al. 1975; Pal and Saxena 1975). In these studies, rhizobial inoculation was a common practice. Ndoye and Dreyfus (1988), in a pot experiment, exposed S. rostrata plants to artificial illumination for 14 h to inhibit early flowering. Pareek et al. (1990) made a field estimate in concrete-based plots with a depth of 30 cm, using the $^{15}$N-dilution technique. The primary objective of the present study was to evaluate N$_2$ fixation by S. speciosa in the 50 days following establishment, using the isotope-dilution technique, compared with that of S. rostrata, an extensively studied green manure plant used for lowland rice and other farming systems (Danso and Kumarasinghe 1990). The ideal reference plant technique largely depends on the choice of a satisfactory non-fixing reference plant (Tropaqualf) with a pH of around 7.3, 0.095% total N, 5.1% organic matter, 94 μg g$^{-1}$ available P, and 0.29 mmol 100 g$^{-1}$ exchangeable K.

$N_2$ fixation by S. speciosa and S. rostrata

Reference plant selection trial. The accuracy of the $^{15}$N-dilution technique largely depends on the choice of a satisfactory non-fixing reference plant (Danso and Kumarasinghe 1990). The ideal reference plant has similar rooting patterns and similar N-uptake profiles to those of S. rostrata. The ideal reference plant technique largely depends on the choice of a satisfactory non-fixing reference plant, which is south of Anuradhapura. The annual rainfall in this region is around 1300 mm, distributed in two crop-cultivation seasons, the Maha or major period, which extends from October to February, and Yala the minor period, which extends from April to July. The soil is an imperfectly drained low humic gley (Tropaqualf) with a pH of around 7.3, 0.095% total N, 5.1% organic matter, 94 μg g$^{-1}$ available P, and 0.29 mmol 100 g$^{-1}$ exchangeable K.

Materials and methods

Field site

The experiments were carried out in a lowland field at the agricultural research centre at Maha Illuppallama, which is south of Anuradhapura in the North-Central province (8°10' N, 80°35' E) of Sri Lanka. Mean annual rainfall in this region is around 1300 mm, distributed in two crop-cultivation seasons, the Maha or major period, which extends from October to February, and Yala the minor period, which extends from April to July. The soil is an imperfectly drained low humic gley (Tropaqualf) with a pH of around 7.3, 0.095% total N, 5.1% organic matter, 94 μg g$^{-1}$ available P, and 0.29 mmol 100 g$^{-1}$ exchangeable K.

In May 1989, a field plot was cleared and seeded with the non-fixing and fixing plants in separate rows, 25 cm apart, with 25 cm between plants, and the soil was kept moist. Fifty days after seeding, six randomly selected plants were carefully uprooted together with the surrounding soil from each row. The soil was washed off and the lengths of apical root and lateral roots were recorded. The plants were oven-dried and dry weights recorded. Ground samples of different plant types were analysed for total N (Kjeldahl method) and the N yield per plant was calculated. In the second step of this experiment, in September 1989, all the reference and test plants were seeded in a similar manner. They were removed at 2-week intervals, oven-dried, weighed, and the N contents determined on ground samples, until 50 days after establishment. The N uptake per plant was calculated and corrected, using the seed N content of each species, and was plotted against time. From these results, the non-fixing plants that were most compatible with the test plants were selected as reference plants.

Field evaluation using $^{15}$N. Both the test and the reference plants must be affected similarly by changes in the environment for the $^{15}$N-dilution technique to be successful (Hardarson and Danso 1989). Therefore, both plants were seeded randomly in the same plots at a spacing of 25 × 25 cm, in November 1989. These plots had a background $^{15}$N label as they were earlier used for $^{15}$N-labelling of the two Sesbania spp. in the N-transfer experiment described below. Thirty-five days after seeding, a fertilizer solution (a mixture of urea and ammonium sulphate) of 15% atom $^{15}$N excess was added to the soil, at a rate of 25 kg N ha$^{-1}$. These plots were kept drained but moist. All plants were harvested 50 days after seeding and were analysed for total N and $^{15}$N enrichment. $^{15}$N analyses of samples prepared using Kjeldahl wet digestion (Axmann et al. 1990) were carried out at the Ministry of Agriculture and Fisheries Ruakura soil and plant research station in New Zealand. The percentage N derived by fixation from the atmosphere (%Ndff) in the test plants was calculated using the following equations:

$$\text{%Ndff} = \left(1 - \frac{\text{atom}_\text{%}^{15}\text{N excess in test plant}}{\text{atom}_\text{%}^{15}\text{N excess in reference plant}}\right) \times 100$$

$$\text{N yield of test plant} = \text{dry matter yield} \times \text{%Ndff}$$

N derived by fixation = N yield × %Ndff

Transfer of N from the two Sesbania spp. to a rice crop

A $^{15}$N isotope label was used in this experiment to trace N derived by the rice crop from the added N source. Field trials were conducted during the Maha season of November 1989 to February 1990. These consisted of two different types of plots, isotope plots of 1 × 1 m for tracing N uptake, and yield plots of 5 × 4 m for monitoring yield responses corresponding to the isotopic treatments. The isotope plots were arranged in a random complete block design with four blocks. Their borders were lined with polythene to prevent the exchange of isotopes between plots. The treatments assigned to the isotope plots are given in Table 1.

$^{15}$N-labelling of green manure species. Eight field plots of 1 × 1 m were situated at some distance from the isotope plots to prevent contamination with $^{15}$N. They were seeded with S. speciosa and S. rostrata separately, each type in four plots, at a spacing of 25 × 25 cm. The plots were maintained for 50 days (September–October 1989), during which period a mixture of labelled urea and ammonium sulphate was applied to provide N to the soil at 45 kg ha$^{-1}$. As the purpose of labelling was to obtain a green manure with a high $^{15}$N enrichment, the amount and label of the N added per application was increased as the plants developed, to synchronize its uptake with the plant growth. The labels of the fertilizers used were 1%, 10%, and 50% atom excess $^{15}$N. At harvest, the plants were uprooted, washed, chopped, and mixed thoroughly. Subsamples were oven-dried at 60°C for 72 h, ground, and stored for later analyses. After the plants were uprooted, the same plots, with residual labelling, were used for the $^{15}$N fixation experiment.

Incorporation of green manures and management of the trial. The fresh biomass of each species in the $^{15}$N-labelling plots was divided into four portions and applied to the replicate plots. Unlabelled green manure was also produced in a similar manner, but without the addition of $^{15}$N fertilizer. The isotope plots were planted on the same day that the green manure was incorporated, with 12-day-old BG 34-8 (3- to 3.5-month variety) rice seedlings, at a spacing of 15 × 15 cm; the seedlings were fertilized with the recommended level of P and K (12.98 g m$^{-2}$ of P$_2$O$_5$ and 8.85 g m$^{-2}$ of K$_2$O). Weeds were uprooted and also incorporated into the soil. Recommended insecticides were applied. At maturity, the rice crop was harvested separately on each plot, and divided into grain and straw, oven-dried, and dry weights recorded. Ground subsamples of the dried materials were analysed for total N and $^{15}$N enrichment as described.